CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

DIFENZOQUAT METHYL SULFATE

Chemical Code # 001930, Tolerance # 00369
SB 950 # 038

January 25, 1999
Revised November 17, 2003

I. DATA GAP STATUS

Combined, Rat:	No data gap, no adverse effect (Chronic/Onco)
Chronic toxicity, dog:	No data gap, no adverse effect
Oncogenicity, mouse:	No data gap, no adverse effect
Reproduction, rat:	No data gap, no adverse effect
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time[1]

Toxicology one-liners are attached.

All record numbers through 123746 (volume 63) were examined.
** indicates an acceptable study.
Bold face indicates a possible adverse effect.
File name: T990125
Original: Kishiyama and Gee, 1/25/99

[1] See notes under Neurotoxicity.

"Reregistration Eligibility Decision (RED): Difenzoquat" was issued by US EPA in September, 1994.

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

** 369-003 004540, "Chronic Oral Toxicity Study in Rats with AC 84777", (D.E. Bailey, Director, Food and Drug Research Labs., Laboratory No. 1626a, 9/19/75). AC 84777 [lot AC-1786-158], purity 98.1%, was mixed with the diet at concentrations of 0, 100, 500, or 2500/5000 ppm and fed to 60 FDRL Wistar rats/sex/group for 104 weeks. Dose was increased after the 30th week (not explained - see volume 061, exhibit 2). Hematology for 6/sex/group at 3, 6, 12, 18 and 24 months. Limited clinical chemistry parameters. 10/sex/group were sacrificed at 13 weeks. Histopathology was performed on the controls and high dose animals and limited tissues of the low and mid-dose animals. Eye exam at initiation, 90 days and termination. Reviewed as unacceptable with insufficient information for assessment. (D. Shimer & J. Christopher, 3/13/85) The study was re-evaluated by Gee, 10/29/98, considering the submission in 369-061 [see below]. The original reviewer mentioned thyroid neoplasia as a finding. The incidence of adenocarcinomas was 4/97 in controls and 6/59 at the high dose. The p value was calculated as 0.124, not significant. Follicular adenoma incidence was 3/97 in controls and 1/59 at the high dose. Although there were some deficiencies in the study (lack of a target organ, no diet analyses [see mouse oncogenicity for stability], limited clinical chemistry/urinalysis), the study has been upgraded to ACCEPTABLE status. NOEL = 500 ppm (body weight). (Gee, 10/29/98)

369-061, Exhibit 2. Response to review of J. Christopher, 3/13/85. The response states that the dose was increased at week 30 to insure a toxic effect. In this study, the effect was decreased body weight at the high dose. This was especially found with the females: Body weight was 91% of control at week 104, 86% of control weight at week 78 and 89% of control female weight at week 52. Body weights for males in the later part of the study were 92 - 93% of control body weight. Although survival was mentioned in the initial worksheet, it was not a reason for considering the study to be unacceptable. Survival at termination was as follows: males: 31%, 32%, 40% and 44%, control through high dose; females: 50%, 50%, 74% and 70%, respectively. No analyses of diet apparently were conducted at the time of the study. Difenzoquat, however, was stable in the diet of mice for two weeks in a study by Hazleton. Only 6 of the recommended ten animals per sex were examined for hematology and clinical chemistry with no treatment-related findings. This was a minor deficiency. With review of the response by the registrant, the study has been upgraded to ACCEPTABLE status. (Gee, 10/29/98)

CHRONIC TOXICITY, DOG

** 063 123746, "One-Year Oral Toxicity Study in Purebred Beagle Dogs with AC 84,777 via Capsule Administration", (C. M. Kelly, Bio/dynamics, Inc., Lab. Project ID 90-3640, 5/6/93). AC 84,777, purity 99.4%, administered via gelatin capsules to 4-6 Beagle dogs/sex/group for one year. Dosage of 12.5 mg/kg/day remained the same throughout the study. Doses of 37.5 and 75

mg/kg/day were lowered to 20 and 30 mg/kg/day at week 5 and the high dose group of 125 mg/kg/day was eliminated by day 9 due to excessive toxicity. Dose levels, mode of test article administration, and diet regimen were modified during the study, to compensate for excessive toxicity/mortality. Other treatment related effects were clinical toxicity, reduced body weight gain, and pathological effects (mostly affecting those that died). Once the doses were adjusted to 20 and 30 mg/kg, the clinical signs, body weight effects and food consumption were similar to controls. NOEL = 30 mg/kg (no macroscopic or microscopic findings in surviving animals.) ACCEPTABLE. (Kishiyama and Gee, 11/3/98)
US EPA considered the NOEL for females to be 20 mg/kg based on weight gain and 30 mg/kg for males.

SUBCHRONIC, DOG

369-017 No record number. "90-Day feeding study in dogs with AC 84777." (G. E. Cox, D. E. Bailey and K. Morgareidge, Food and Drug Research Laboratories, No. 1680, 9/28/73). AC 84777, 98.1%, was fed to beagle dogs, 6/sex in the control and 4/sex in test groups, at 0, 100, 500 or 2500 ppm, six days per week for 13 weeks. No changes were reported in hematological, biochemical, ophthalmological or pathological examinations due to treatment. UNACCEPTABLE (dose selection not justified). No worksheet. (Gee, 12/15/98)

ONCOGENICITY, RAT

See combined, rat.

ONCOGENICITY, MOUSE

369-017 004544, "Eighteen Month Carcinogenicity Study on AC 84777", (P. C. Underwood and A. S. Tegeris, Pharmacopathics Research Laboratories, 2/26/75). AC 84,777, purity not stated, was admixed with the feed at concentrations of 0, 0, 100, 500 and 2500 ppm and fed to 60 CD-1 mice/sex/group for 18 months. Ten animals per cage. At 6 and 12 months, 5/sex/dose were given an examination and histopathology performed. At 18 months, 10/sex from the high dose group and the two controls were given a complete histopathological examination. No analysis of the diet, incomplete presentation of tissues examined - inadequate pathology. UNACCEPTABLE. Insufficient information for assessment. (J. Christopher, 3/13/85).

** 054, 061 075455, 116095 "Chronic Dietary Toxicity and Oncogenicity Study with AC 84,777 in Mice", (K. M. MacKenzie, Hazleton Laboratories America, Inc., HLA 6123-145, 6/23/89.) AC 84,777, purity 98.7%, admixed with the feed at concentrations of 0, 200, 500 or 1000 ppm was fed to 65 Crl:CD®-1(ICR)BR mice/sex/group for 79 weeks. Doses were equivalent to: M - 26.9, 69.4 and 150.1 mg/kg/day; F - 39.7, 97.9 and 202.4 mg/kg/day. Hematology was performed with 10/sex/group at week 53 and at termination. Ten per sex per group were sacrificed at week 53 and examined. Survival was comparable for all groups. Body weight was lower for the high dose group with males being 87% of controls and females, 92%. No other treatment related effects were reported. No evidence of oncogenicity. ACCEPTABLE as an oncogenicity study only. Systemic NOEL = 500 ppm based on lower body weights. Chronic portion of study is unacceptable (serum chemistry, urinalysis and ophthalmological examination were not conducted). (Kishiyama and Gee, 10/8/98).

REPRODUCTION, RAT

** 067, 068 147683, 147687 Schroeder, R. E. “A Two-Generation Reproduction Study with AC 84,777 in Rats.” (Huntington Life Sciences, Laboratory Project ID 93-4066. May 16, 1996). AC 84,777, purity 99.4%, admixed with the feed at concentrations of 0, 150 [75], 1500, and 3000 ppm was fed continuously to P1 parental animals from pre-mating (10 weeks) to weaning of the F1a pups. The low dose was lowered to 75 ppm for the F1b litter and for F1 parental animals (pre-mating was 11 weeks, 1 litter (F2)) The F1 breeders were selected from the F1a litters. **Reproductive NOEL = 75 ppm** (lower body weight, no effects on mating, gestation length, live birth index, viability index, weaning index). Pup body weight was lower prior to weaning for mid and high dose F1a, F1b and F2 litters. Body weight was 5-8% lower and associated with 14-17% reduced body weight gain for high dose P1 parental animals. Body weight gain was also suppressed 10-14% for males only in F1 mid and high-dose groups. Parental NOEL = 75 ppm. No adverse reproductive effects. 369-068 147687 was a pilot study. ACCEPTABLE. (Kishiyama, Gee, 10/9/98)

017 004545, "Three-Generation Reproduction Study in Rats", (F. E. Reno, Director, Hazleton Laboratories, Inc., Project No. 362-147, 10/14/74). AC 84,777, purity not stated, was admixed with the feed at concentrations of 0, 500 or 2500 ppm and fed through three successive generations to 10 male and 20 female Charles River rats/group/generation. Possible NOEL = 500 ppm based on body weight. UNACCEPTABLE. No analysis of feed, only two doses, body weights recorded monthly, necropsy on 1/3 of pups, histopathology deficient in numbers and sacrifice times, no histopathology on adult breeders. Major variances and insufficient information. (J. Christopher, 3/14/85).

TERATOLOGY, RAT

** 369-058 088214, "An Oral Developmental Toxicity (Embryo-Fetal Toxicity/Teratogenicity) Study with AC 84,777 in Rats", (E. A. Lochry, Argus Research Laboratories, Lab Report No. 101-008, 3/22/90). AC 84,777 technical, purity 99.4%, administered by gavage once daily at concentrations of 0 (deionized water), 30, 60, 120 and 240 mg/kg to 25 mated Crl:CD®(SD)BR female rats/group on days 6 through 15 of gestation. An increased incidence of excessive salivation and reduced feed consumption was observed at 120 and 240 mg/kg/day. Maternal body weight was lower on several occasions during dosing. Maternal NOEL = 60 mg/kg/day. There was no evidence of developmental toxicity. Developmental NOEL = 120 mg/kg/day (lower fetal weight, although not statistically significant.) ACCEPTABLE. (Kishiyama and Gee, 10/8/98)

369-017 004546, "Teratology Study in Rats", (F. E. Reno, Director, Hazleton Laboratories Inc., Project No. 362-150, 7/22/74). AC 84,777, purity 98.1% (assumed), admixed with the feed at concentrations of 0, 500 and 2500 ppm and fed to 13 - 18 females/group during days 6 through 15 of gestation. UNACCEPTABLE. Insufficient information. Not upgradeable (no maternal toxicity, too few animals evaluated, other major variances). (J. Christopher, 3/14/85).

TERATOLOGY, RABBIT

** 51 069626, 069628, "A Teratology Study with AC 84,777 in Rabbits", (K. M. MacKenzie, Hazleton Laboratories America, Inc., Study No. 6123-114, 12/14/84). AC 84,777, purity 99.1%, was

administered by oral gavage at nominal doses of 0, 50, 100, or 250 mg/kg to 18 artificially inseminated New Zealand White rabbits/group on Days 7 through 19 of gestation. Mortality was 61% for the high dose group with 2 deaths due to dosing error. Nominal maternal NOEL = 100 mg/kg (mortality, clinical signs) Nominal developmental NOEL = 100 mg/kg (centra abnormalities of the vertebra in 1/4 litters at 250 mg/kg - not statistically significant). Fetal incidence was 0/119 in controls and 2/17 in the high dose (statistically significant, p = 0.015), a dose at which there was significant maternal toxicity. No adverse effects. ACCEPTABLE. (Kishiyama and Gee, 11/6/98)

051 069627. "A Range-Finding Study with AC 84,777 in Rabbits", (K. M. MacKenzie, Hazleton Laboratories America, Inc., Lab Report No. 6123-113, 7/23/84 [note: study conduct was reported as 2/1/84-3/1/84]). AC 84,777 (difenzoquat), 99.1%, was administered via gavage at doses of 0, 5, 10, 25, 50 or 100 mg/kg to 5 artificially inseminated New Zealand White female rabbits/group during Days 7 through 19 of gestation. Survival was 100% for all dose groups except, 80% and 60% for the 25 and 100 mg/kg dose groups, respectively; however, all deaths were attributed to dosing error. No treatment related effects reported. (no worksheet). (Kishiyama and Gee, 11/6/98).

GENE MUTATION

** 369-051 069629, "Bacterial/Microsome Reverse Mutation (Ames) Test on CL 84,777", (J. S. Allen, American Cyanamid Company, Lab Report No. Gtox Volume 4, pages 1-26, 8/21/84). CL 84,777 [difenzoquat], purity 99%, was tested at concentrations of 0, 50, 158, 500, 1581 or 5000 ug/plate, with S. typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538, and E. coli WP-2 $uvrA^-$ in the presence and absence of Aroclor 1254-induced rat liver S-9 Mix. Triplicate plates per concentration with repeat trials. Mean number of revertants did not increase in either the initial or repeat tests. ACCEPTABLE. (Kishiyama and Gee, 11/6/98)

CHROMOSOME EFFECTS

** 369-051 069630, "In Vitro Chromosomal Aberrations in Chinese Hamster Ovary Cells with AC 84,777 ", (T. Cortina, Hazleton Laboratories America, Inc. Laboratory Report No. 362-172, 1/30/85). AC 84,777, purity 99.1%, at concentrations of 0, 100, 333, 1000, 3330 and 10000 ug/ml without metabolic activation for 3, 8 or 12 hours and at 0, 33, 100, 333, 1000 and 3330 ug/ml with metabolic activation was evaluated at 3, 8 and 12 hours after a two hour treatment period for clastogenic potential on Chinese Hamster ovary cells. NOTE: Actual dose levels were approximately 20-40% lower than nominal. A repeat test at 3 hours exposure time showed no significant increase in chromosomal aberration frequency not confirming the finding in the initial trial without activation at 3330 ug/ml, a cytotoxic level. No increase in aberrations at 8 or 12 hours. Mitomycin C and cyclophosphamide as positive controls for the 12 hour harvest only. ACCEPTABLE. (Kishiyama and Gee, 11/9/98)

369-017 004547, "Dominant Lethal Study in Rats", (D. E. Bailey, Director, Food and Drug Research Laboratories, Inc., Lab No. 2088, 9/3/74). AC 84777, purity not stated, was admixed with the feed at concentrations of 0, 500 and 2500 ppm and fed to 15 weanling male rats/group for 60 days. The treated males were then mated (1:1) with untreated virgin females and the same mating procedure repeated for 8 consecutive weeks. Corpora lutea, implant sites, resorptions, live fetuses, body weights and food consumption were evaluated. Insufficient information. UNACCEPTABLE. Not upgradeable. No positive control group data presented, no justification for dose selection with

no toxicity reported. (D. Shimer and J. Christopher, 3/14/85, updated by Gee, 10/7/98).

DNA DAMAGE

** 369-051 069631, "Rat Hepatocyte Primary Culture/DNA Repair Test", (T. R. Barfknecht, Study Director, Pharmakon Research International, Inc., Lab No. PH 311-AC-003-84, 1984). AC 84,777 at concentrations of 0 (DMSO and untreated), 0.8, 2.6, 8.0, 26.0, 80.0, 266, 800, 2666 and 8000 ug/well were evaluated after 18-20 hours exposure of male Fischer 344 rat hepatocytes (1 x 10^5). AC 84,777 concentrations of 266.0 ug/well and above were cytotoxic. Eighty ug/well and lower doses did not increase the mean net number of grains/nucleus. Sixty cells per concentration were evaluated from three coverslips. 2AAF was active as the positive control. ACCEPTABLE. (Kishiyama and Gee, 11/9/98)

NEUROTOXICITY

Not required at this time. Note: In the RED issued by US EPA in 1994, both an acute and a 90-day neurotoxicity study in the rat were being required. If these studies are positive, a developmental neurotoxicity study will be required. (Gee, 12/16/98)

369 - 070 207915 “Support for waiver from acute neurotoxicity screening battery (81-8-SS) and 90-day neurotoxicity screening battery (92-7-SS).” A cover letter from L. L. Whatley, dated October 14, 2003, BASF, regarding the two neurotoxicity studies required in the RED of 1994. These studies are no longer being required by US EPA. The volume also contains a 6-page document discussing the 1-year dog study and the rat developmental study, both of which indicated clinical signs. These signs were attributed to the irritation of the gastrointestinal tract rather than to neurotoxicity. No worksheet. (Gee, 11/17/03)